Abstract No. boes153

Structure of the Ligand Binding Domain of a Type II TGF-beta Receptor

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Beamline(s): X9B

Introduction: TGF-beta is a pleiotropic, multifunctional cytokine with potent immunoregulatory properties. The role of TGF-beta has been established in many processes including inflammatory response, carcinogenesis and oral tolerance as the primary inhibitory cytokine. Our current goal is to gain structural insights into the molecular recognition between the cytokine and its receptors and thus achieve an understanding in the activation of cells bearing the receptor.

Methods and Materials: After brief soaking in precipitant solutions containing 25% glycerol, crystals were flash frozen at 100 K. X-ray diffraction data from single crystals were collected using an ADSC Quantum IV CCD detector at the X9B beam line of the National Synchrotron Light Source (NSLS), Brookhaven National Laboratory and processed with HKL2000. The crystals belong to a space group $P2_12_12_1$ with cell dimensions *a*=35.471, *b*=40.658 and *c*=76.154 Å, contain one monomer of TGFβ-RII per asymmetric unit, and diffract to 1.1 Å. MAD dataset was collected from the crystals soaked in $HgCl_2$ at the X9B beamline (NSLS). Three Hg sites were found by SOLVE. After density modification including solvent flipping, the electron density of was traced using ARP/WARP autotracing program. Model adjustments and rebuilding were done using program O. The positional and individual *B*-factor refinement was carried out using a maximum likelihood target function of CNS v1.0. The refined model consists of residues 1-105 of TGFβ-RII sequence, residues 106-110 of C-terminal sequence are disordered. A well-defined glycerol molecule and 165 water molecules were located in the electron density.

Results: Overall structure of TGFβ-RII resembles that of activin receptor II (ActRII) extracellular domain. It shows so-called three-finger toxin fold that has been observed for proteins having a common pattern of eight cysteins forming four disulfide bonds. TGFβ-RII has twelve cysteins forming six disulfide bonds, four of them C3-C36, C29-C53, C73-C88, and C90-C95 are conserved among proteins displaying a three finger toxin fold. Three fingers are formed by pairs of β-strands β1-β2, β3-β4, and β5-β6. Although overall fold of TGFβ-RII is rather similar to ActRII, there is a very prominent difference. First finger in TGFβ-RII structure folds tightly between the second one and C-terminus, whereas in ActRII structure it flips away from other two fingers. The tightly packed first finger in TGFβ-RII structure forms an antiparallel β strand with C-terminus and stabilized by interactions with β5-strand of the second finger by forming an antipalallel β-strand on the short stretch. Overlay of the TGFβ-RII and ActRII structures shows remarkable 35 Å difference in the position of their first fingers.

Conclusions: We have determined the crystal structure of the extracellular ligand binding domain of a human type II TGF-beta receptor to 1.1 angstrom resolution. The structure belongs to a three-finger toxin fold and resembles to that of the type II activin receptor.

Acknowledgments: We thank C. Hammer for mass spectroscopy measurements; J. Johnson and Z. Dauter for help with X-ray synchrotron data collection; this work was supported by the intramural research funding from the National Institute of Allergy and Infectious Diseases.

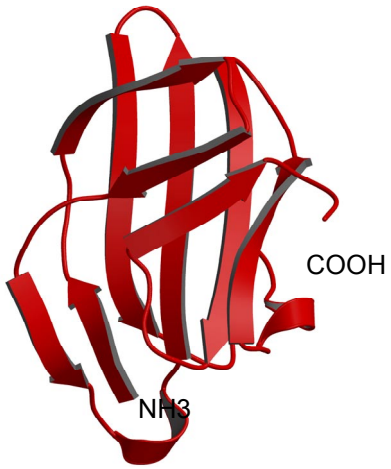

Figure 1. Crystal structure of the type II TGF-beta receptor ligand binding domain.